RELATIVE EFFICIENCY OF FOUR APHID SPECIES IN TRANSMISSION OF BARLEY YELLOW DWARF VIRUS AND USE OF DIFFERENTIAL VARIETIES IN STRAIN IDENTIFICATION

by

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INTRODUCTION

Early investigations on barley yellow dwarf virus (BYDV) revealed that several aphid vectors could transmit this virus but their relative efficiencies varied (34). Oswald and Houston (34) believed that the apple grain (AG) aphid, Rhopalosiphum fitchii (Sand.) (they referred to it as R. prunifoliae) and the English grain (EG) aphid, Macrosiphum granarium (Kirby) (= M. avenae) were most important. Later studies by Toko and Bruehl (64, 65) and Rochow (36, 37) revealed that the efficiency with which these aphid species transmitted BYDV depended on the strains of the virus. In Washington (8, 64, 65) the AG aphid transmitted certain strains of the virus efficiently but only the EG aphid was an efficient vector of strains more prevalent in New York State (36). Furthermore, the greenbug (GB), Toxoptera graminum (Rond.) (= Schizaphis graminum, Rond.) which was earlier not considered to be a very important vector of BYDV, appeared to be the most important vector in the 1959 epidemic in at least certain areas (2, 27, 28, 49). Rochow (41) later reported that physiologically different strains of GB differed in their ability to transmit BYDV. Recently Sakesna et. al. (47) offered positive evidence to emphasize the important role of GB in field transmission of BYDV.

Vector-specific isolates have been encountered in different areas (8, 36). So far, AG, EG, and the corn leaf (CL) aphid, Rhopalosiphum maidis (Fitch) - specific strains have already been reported. Bruehl (11) had cited at least 8 aphid species as important vectors of BYDV. However, all of them were not equally effective. Newer vectors are being discovered (53). It will not be surprising if more vector-specific strains are

reported. The presence of vector-specific strains and other vector virus relationships further complicate the study. The ability of virus strains to be transmitted by several rather than 1 or a few vector species favors the chances of their survival and consequently their spread in the field. Fluctuations in aphid population from year to year or season to season requires critical observations.

Because of the considerable variability in the relative importance of the different species as vectors of BYDV strains in different areas, it was thought necessary to evaluate the relative importance of several aphid species in relation to the problem of BYDV in Kansas, and to test the efficiencies with which common Kansas isolates of BYDV could be transmitted by 4 common grass feeding aphids. Using differential small grain varieties an attempt was also made to identify strains of BYDV in Kansas.

REVIEW OF LITERATURE

While discovering barley yellow-dwarf virus (BYDV) disease, Oswald and Houston (32, 34) recognized that this was a "yellows type" of virus disease which was readily transmitted by at least 5 aphid species (32, 33, 34). Later workers have added seven more vectors (11, 23, 44, 53). All the known vectors are listed in Table 1. The BYDV is spread by aphids and apparently in no other way. At least up to now, all efforts to transmit the virus by other means have failed (8, 34, 63). The multiplicity of vectors seems more than adequate to insure dissemination of the virus but the situation is rendered more complex by the existence of vector specificity. Oswald and Houston (32) found that BYDV infected barley, wheat and oats. Their later studies (35) and those of Bruehl and Toko (9) and

Table 1. Known vectors of Barley Yellow Dwarf Virus.

Aphid	Reference	
1. Rhopalosiphum fitchii, apple grain aphid	Bruehl & Toko	1955
R. prunifoliae (Fitch), apple grain aphid	Oswald & Houston	1953
R. padi-fitchii complex, apple grain aphid	Orlob	1959
2. Rhopalosiphum padi (L.), the bird cherry oat aphid	Watson & Mulligan	1957
3. Rhopalosiphum maidis (Fitch), corn leaf aphid	Oswald & Houston	1953
4. Rhopalosiphum poae (Gill), blue grass aphid	Orlob	1959
5. Macrosiphum dirhodum (Walker), grass aphid	Oswald & Houston	1953
Metopolophium dirhodum (Walker), rose grain aphid	Watson & Mulligan	1957
6. Macrosiphum granarium (Kirby), English grain aphid	Oswald & Houston	1953
M. avenae (Fab.), English grain aphid	Slykhuis et. al.	1959
Sitobium avenae (Fab.), English grain aphid	Watson & Mulligan	1960
7. Macrosiphum avenae s.sp miscanthi Takahasi	Butler et al.	1960
8. Necmyzus circumflexus (Buckt.), crescent-marked lily aphid	Watson & Mulligan	1960
9. Toxoptera graminum (Rond.), greenbug	Oswald & Houston	1953
Schizaphis graminum (Rond.), greenbug	Smith	1963
10. Sitobium fragariae (Walker), rubus aphid	Watson & Mulligan	1957
11. Myzus persicae (Sulz.), the green peach aphid	Smith	1963
12. Sipha agropyrella (H.R.L.), the quack grass aphid	Smith	1963

Names indented under 1, 5, 6 and 9 have been used by some authorities to identify species here referred to only by the name numbered.

Watson and Mulligan (69) showed some 84 plant species to be susceptible to one or more isolates of the virus.

Aphids are the 'precision tools' for transmitting the virus. However, not all aphid vectors are equally effective in their ability to transmit the virus (8, 10, 11, 28, 31, 34, 37, 39). The various species of aphids differ not only morphologically but also in their physiological behavior, which influences their effectiveness as vectors. They differ in their abilities to transmit a given strain of BYDV; in their preference for different grasses; in their rate of multiplication under different temperatures; in their feeding and flight habits and in their overwintering abilities (11). The main vector may be different in epidemic years and different in farming areas during the same year (39).

Bruehl and Toko (8) reported that the AG aphid was the major vector of BYDV in Washington. They further noted that the plants affected by means of the AG aphid were more severely stunted and developed more prominent symptoms and concluded that the difference was not quantitative and that the AG aphid transmitted a more virulent virus than other aphids. Rochow (36) observed that the EG aphid was more important in New York and other northeastern states. In an extensive study on the abilities of the EG aphid, the AG aphid, the CL aphid, and the GB he noted that the GB was the least efficient vector. This was rather surprising since this aphid had been successfully used in several transmission studies (16, 28, 33). However, in 1959, the GB was considered by many workers to be the principal vector in some areas (2, 27, 28, 49). Sill et. al. (49) had observed that the aphid populations, particularly the GB were very high in eastern Kansas in both the fall of 1958 and the spring of 1959 and believed that the GB

played a major role in yellow-dwarf damage. The GB is less of a roamer than other grass aphids. Hence, in most seasons it might be less important as a vector of BYDV. Body (16) reported that GB collected in Kansas were efficient vectors of a strain of BYDV used in his study. In 1962, Medler (24) reported trapping of the GB in sufficient number at Manhattan (Table 2). Recently Saksena et. al. (46) emphasized importance of the GB in field transmission of BYDV and its effectiveness as a vector of BYDV isolates collected in Kansas. Rochov (41) reported that 'physiological specialization' existed among the GB in transmission of BYDV, and this could account for the low recovery of virus in the former tests. This is important and consideration needs to be given to this new variable, i.e. specialization among collections of an aphid vector. Several kinds of variations among isolates of a virus are known and it is not surprising that variation should also be found among collections of a vector. There seems no reason why such variability should not be important in nature.

Orlob and Arny (30) studied the transmission of BYDV by different forms of the AG aphid and discovered that all the developmental stages of a vector are not necessarily potential vectors. They concluded that the vector specificity rests with the characters which are genetically fixed.

Stubbs (58) found that individual cultures of Myzus persicae (Sulz.) varied in their ability to transmit the virus and that selected cultures retained their characteristics. He postulated that inactive insects might occur more frequently in vector species than it is at present realized and could account for much of the variability which characterizes virus-vector relationships.

Table 2. The number of aphids collected under the North Central States cooperative project on aphid dispersal.[1] Manhattan, Kansas, 1962 (average of 2 traps).

Aphid species	April		May												June
	26	28	2	4	6	8	10	12	15	17	19	22	24	29	4
Aphis rumicis			2	2											
Aphis sp.			1				2	3	1						
Chaetophorus sp.							3	1							
Eriosoma lariqerum										1					
Hyadaphis atriplicis							1		1					4	9
Macrosiphum granarium								1	1			3	1		
Macrosiphum pisi	3	4	3	3	9	10	8	17	11	8	9	8	5	1	1
Mysolcallis punctata									1						
Myzus persicae		1		1			1	3							
Pemphigus populitransversis				1					1						
Rhopalosiphum fitchii	1			1				1	1						
Rhopalosiphum maidis							1					1			
Rhopalosiphum pseudobrassicae			1	1			1							1	
Therioaphis rumicis								1							
Therioaphis trifolis							1				1				
Toxoptera graminum		3	5	4	9	8	3	6	10	5		1			

[1]Sorting and identification of aphid collections done by J. T. Medler, Professor, Department of Entomology, University of Wisconsin, Madison 6 (24).

Saksena et. al. (48) recently reported the transmission of a strain of BYDV by 4 biotypes of the CL aphid, and found that biotype KS-2 was highly significant over the other 3 biotypes in its transmission pattern as well as in its transmission percentage. No statistical difference was observed amongst the biotypes KS-1, KS-3 and KS-4. All the four biotypes were, however, quite efficient as vectors and are potentially important enough to cause field infection.

The time needed for the bulk of aphids to establish satisfactory feeding relations is variously estimated from a few minutes to several hours. Oswald and Houston (34) demonstrated that upon becoming viruliferous aphids remained so for life and that single viruliferous aphids could infect the plant. Nymphs were virus free at birth. Once they obtained virus from a host plant, they might continue to transmit it to the plants. Toko and Bruehl (66) reported that nymphs serve as effective vectors as adults. Watson and Mulligan (69) found that virus persisted in the vector through moulting.

Watson and Roberts (68) suggested the terms persistent and non persistent to designate the two types of insect-transmitted plant virus. Other workers (11, 34, 49) are in general agreement that yellow-dwarf virus persists in its vectors; that some of the strains of the virus are better adapted to transmission by one aphid than another; that many strains are adapted to transmission by several aphid species and that there is no interference among strains of yellow dwarf virus within the host or within the aphid (38, 65). However, details of acquisition and inoculation feedings are less well established for all the vectors. Rochov (37) using single aphids found that an acquisition period of one hour resulted in

occasional transmission but demonstrated the persistence of virus in the AG aphid and the EG aphid for life following a 24 hour feeding. He recommended that at least 24 hour acquisition feeding and 24 hour inoculation feeding periods should be given in routine work to get the best results.

Watson and Mulligan (69) reported that the bird-cherry oat aphid, Rhopalosiphum padi (L.) must feed for more than one day to acquire and more than one day to inoculate, if transmission is to occur in high percentage. Toko and Bruehl (64) working with two strains of virus and a single aphid collection failed to obtain transmission when the AG or the EG aphids were allowed acquisition feedings of 16 hours or less. Acquisition feeding of 24 hours was adequate. Likewise they failed to obtain transmission if inoculation feedings were short. Using the AG aphid in an acquisition feeding period of 10 minutes, Orlob (28) also failed to get transmission. But when the aphid was allowed at least a week on the source plant, inoculation feeding was successful in 30 minutes.

Watson (67) studied the transmission of sugar beet yellow virus by the aphid Myzus persicae (Sulz.) and found that the efficiency of the vector in transmitting the virus increased greatly with the increased feeding time on the infected plants. The behavior of sugar beet yellow virus was comparable to that of curly top virus of sugar beet in which infectivity also persists for an indefinite period in the vector and increases with increasing feeding time on infected and healthy plants. The transmission of the curly top virus by the leaf hopper, Circulifer tenellus (Baker) differed fundamentally from the transmission of such viruses as aster yellows, rice stunt, clover club leaf and wound tumor virus.

The evidence reported by Freitag (17) and Bennett and Wallace (6) seemed to indicate a lack of multiplication of curly top virus in the vector.

The extremely short periods of time necessary for the aphid vectors of some viruses to effect transmission from a diseased to a healthy plant have been difficult to interpret. Sylvester (59) transmitted the sugar beet mosaic virus by the green peach aphid from a diseased plant to a healthy plant in a short period of 42 seconds. The short feeding time involved seemed to preclude the possibility of the virus being taken up into the body of the aphid and returned to the plant. These results suggested that the virus was merely taken up into the mouth parts and immediately returned to the plants.

Watson and Roberts (68) demonstrated that starving aphids, preceding a short feeding time on diseased plant greatly increased the efficiency of transmission. If the feeding period on a diseased plant was increased to an hour, the beneficial effects of starvation was lost. This does not support the hypothesis that aphids transmit the virus by mere external mechanical contamination of the mouth parts.

Sylvester (61) reported that, in general, with semi-persistent and persistent viruses, the process of acquisition and inoculation was relatively slow, indicating that the aphids must penetrate rather deeply into the plant tissues either to acquire virus or to inoculate. Usually the process of acquisition was rather slower than that of inoculation, perhaps meaning that areas of active virus concentration were more localized than were the areas in which infection can be initiated.

Simons (50) observed that the acquisition threshold period for the adult pea aphid was found to lie between one to two hours, while the

inoculation threshold period was between 15 to 20 minutes. The effect of length of acquisition feeding on the rate of pick up was almost linear, the effect of the length of the test feeding on the rate of transmission was logarithmic. He found a positive correlation between the length of the acquisition feeding and the length of the retention of the virus. Post acquisition feeding starvation for periods up to 24 hours produced no effect on transmission. The nymphs showed a shorter mean latent period than the adults and it was proposed that this might be a reflection of the differences in vector efficiency.

Anderson (3) found that Macrosiphum geranicola (Lamb.) required between two and three hours to acquire the red leaf virus from a diseased plant but only between 10 to 15 minutes to infect the healthy ones. Forcing infective individuals of M. geranicola to fast for long periods after acquiring the virus did not affect their infectivity, nor did fasting before the acquisition feeding cause aphids to acquire greater infectivity or to become infective faster.

In almost all the viruses that have been studied intensively, strains have been encountered. BYDV certainly proved no exception and exploratory research to date has proved the existence of great variations within the BYDV. Strain differences in BYDV have sometimes been expressed by the production of quantitatively different symptoms on a given host under similar experimental conditions. The genotype of the host influences the symptom expression not only in all catagories, such as symptomless carriers, tolerant, susceptible resistant, etc., but also in such ways as the nature of discoloration and degree of stunting.

Clinch and Loughnane (13) separated the strains of aphid transmitted sugar beet yellows virus on the basis of symptoms expressed by the hosts. The mild yellows strain did not protect against the etch yellows strain. Strain differentiation was achieved in many cases by the use of differential symptom expression on common hosts. Webb et. al. (70) separated 4 strains of the potato leaf roll on the basis of the symptoms produced on one host, Physallis floridana. In studies on BYDV, these methods were also logically used, since the differential symptoms incited by various isolates had been noted earlier. Even in their initial studies, Oswald and Houston (35) observed that BYDV had a wide host range in the grass family and some grasses exhibited typical yellow dwarf symptoms of stunting and either yellow or red discoloration, while others showed no symptoms but proved to be symptomless carriers of the virus.

Bruehl and Toko (9) observed that isolates differed in their ability to infect different grass species and genera and in symptoms they produced. They found that Bromus commutatus was red-purple when infected by one strain of BYDV and severly stunted and bluish green when infected by a second strain.

Takeshita (63) reported that some of the BYDV isolates differed in virulence and caused only moderate stunting and mild leaf symptoms on highly susceptible barley and oat varieties. He also observed that the incubation period of the mildly virulent isolate was 3-7 days longer than that of the highly virulent isolate.

The methods that could be used to separate the strains of BYDV are somewhat limited, since the virus is not mechanically transmitted, no local lesion host has been found and the virus itself has not been purified.

Allen (1) made a detailed study on the differentiation of strains of the BYDV. Upon inoculation of 31 cereal varieties with BYDV isolates, he observed varietal differences in the symptom expression and selected 4 differential hosts, namely 3 barley varieties (Black Hulless, Atlas 46 and Rojo) and 1 oat variety (Coast Black) and tested 43 virus isolates. Allen (1) distinguished 16 virus strains based on the ability of the virus to cause stunting and discoloration of the hosts; the differences were statistically significant. The strains were grouped for convenience into 7 types based on their ability or inability to cause discoloration in each differential host. It was found that mixtures of these strains could be produced by simultaneous inoculations and that mixtures could be separated by aphid transfer to different hosts. No positive cross protection was observed between the strains.

Kunkel (22) showed that certain leaf hopper borne viruses, namely the type variety of aster yellows and the celery yellow strain, cross protect in the vector. Black (5) however, had theorized that it might be that this criterion was applicable only to those cases where the virus multiplied in the vector. Such multiplication had been demonstrated for the type variety of aster yellows virus and presumably holds good for the celery yellow strain.

Giddings (19) studied the inter-relationship of virus strains of sugar beet curly top virus, but did not find any evidence that one would immunize the host against infection by any of the others. The lack of cross protection in the vector between virus strains of curly top virus (6) might be related to the absence of multiplication of that virus in its leaf hopper carrier. Cross protection in the vector might prove to be

not only the indicator of certain relationships of the virus but of virus multiplication in the vector as well. Whether or not the absence of cross protection among the strains of BYDV is an evidence for the lack of multiplication of the virus in the vector needs further study.

Toko and Bruehl (65) reported that cross protection tests failed to show interference between 2 vector specific virus entities. While they observed that introduction of 2 strains in the same host did not alter the vector specificity, Rochow (38) reported quite different results. When the plants were doubly infected with relatively vector specific strains the EG strain was recovered as introduced; the AG aphid, however, recovered virus subsequently transmissible by it and also by the EG aphid. This difference of behavior of mixed strains has not so far been explained.

A comparison of the host ranges determined in Washington (9) and California (35) revealed not only that they were different, but two strains from Washington also differed from each other in their host ranges. Oswald and Houston (35) reported that of the 36 species proved to be hosts, 20 of the grasses exhibited typical yellow dwarf symptoms, while 16 species showed no symptoms but were symptomless carriers of the virus. Smith (51) reported that one strain of BYDV produced moderate infection on oat varieties, Saia and Fulgum, which had been described as field resistant to the virus in Illinois. Although the isolates differed in their host ranges, it was not clear how much of the difference actually reflected the variability among the virus isolates and how much was based on other factors.

The differing host range of different strains of virus, however, should not be over emphasized. For example, Yu et. al. (71) who described a virus disease of foxtail millet, Setaria italica (L.) Beaux.) that appears

similar to BYDV disease, might have placed undue emphasis on the host range in identifying the virus. They considered the virus distinct from BYDV mainly because it was transmitted to 4 hosts found to be immune to BYDV by Oswald and Houston (35). The limited usefulness of this criterion is illustrated by the fact that two of these hosts, (i.e. Digitaria sanguinalis and Zea mays) had been found to be susceptible to other isolates of the BYDV. Marked genetic variation does exist within many grass species. In some cases, the differences, probably due to differences in the host rather than virus, could confuse the situation. Rochow (42) however, obtained seed used in the Washington study and subjected plants derived from it to different strains of the virus. His comparison of the host range showed that the different strains of the virus might differ in host ranges at the genus and species level.

In Washington, Bruehl and Toko (8) reported that with a single virus collection, the AG aphid was more efficient than the EG aphid. Speculations were ripe at this stage to suspect the existence of vector specific strains. Subsequently Toko and Bruehl (65) studied 34 isolates and differentiated two vector specific strains which produced typical symptoms of barley yellow dwarf on oats, wheat and barley; these included mottling, discoloration and leaf serration. They found this quality of vector specificity to be quite stable and persisted in serial transfers. Cross protection tests failed to show interference between the two virus entities even when the symptoms induced by the first strain were visible before the second was introduced. They also reported that acquisition feeding of 24 hours followed by inoculation feeding of 4-8 hours was necessary for infection to be more than rare. Lengthening either feeding

period increased the efficiency of transmission by both the vectors.

Rochow (36) made extensive studies with several isolates of BYDV and reported that majority of his isolates were vector specific, transmitted by the EG aphid, and rarely or not at all by the AG aphid. He also reported some AG-specific strains which were rarely transmitted by the EG aphid.

In another study, Bruehl (10) compared the AG aphid and the EG aphid collected in New York and Washington and observed that the Washington collection, for the most part, had little or no vector specificity in regard to these two aphid species. Aphids from New York were equal vectors to those of Washington, being in no way distinguished in the test. The prevalence of vector specific strains at New York and their rare occurrence in Washington suggested possible regional differences in the virus complex, or their vectors. This led to the exchange of non viruliferous aphids and parallel studies at New York (37) and Washington (10), which still verified the presence of vector specificity in New York and its scarcity in Washington.

Similar specificity was observed by Rochow (39) who reported differential transmission of BYDV from field samples by means of 4 aphid species. Recovery of the virus by different aphid species frequently varied with areas in which samples had been collected. Predominant transmission from samples from Mississippi, Texas, Pennsylvania and New York was by the EG aphid only. The AG aphids were most effective in transmission of samples from California and Illinois, whereas the CL aphid was most efficient only from samples from Florida.

Rochow (42) studied 4 vector specific isolates and reported that the vector specificity remained essentially unchanged after a total of 129 transfers that included 11 serial transmissions of each isolate. In some cases, however, occasional transmission by 'non vector' aphids occurred; such transmission of AG-isolates by the EG aphid were more common than were transmission of EG isolates by the AG aphid. The observed vector specificity of both kinds of isolates was considered to be relative and not absolute, since the virus was occasionally transmitted by a 'non vector' which appeared to be a specific isolate introduced into the source plant and not a mutant or other selection of virus from it. Although the AG aphid transmitted isolates were considered to be strains of BYDV reported from other areas, the EG aphid transmitted isolates appeared to represent a strain of virus common in New York but different from those that were common in other areas of the United States.

Rochow (40) identified 2 strains of BYDV on the basis of transmission by the EG aphid, and the oat bird cherry aphid, Rhopalosiphum padi. One strain was transmitted efficiently by R. padi but rarely if at all by the EG aphid. The other was transmitted by the EG aphid, but rarely by R. padi. The vector specificity of such strains had been absolute in some tests and relative in others. In all cases, however, clear differences between the two virus strains had been shown. Later Rochow (43) reported another strain of BYDV transmitted specifically by the CL aphid. These results are considered as evidence for at least three vector specific strains of BYDV in the field; for the relative, not absolute nature of vector specificity, and for specificity during the transfers of virus strains in the greenhouse.

Smith (47) tested different aphid species in relation to the transmission of BYDV and reported R. padi to be the most efficient vector of the majority of the isolates of BYDV in Canada. However, two isolates transmitted efficiently by the EG aphid and Metopolosiphum dirhodum were not transmitted by R. padi, the AG aphid, the CL aphid and the GB. He also reported Myzus persicae and Siphe agropyrella (H. R. L.) as vectors of BYDV for the first time.

Smith and Richards (54) reported that R. padi which appeared to be a common and efficient vector of BYDV, had frequently been mistaken for R. fitchii. They also proposed a dosage concept to explain the vector efficiencies of R. padi and R. fitchii and suggested that the strains of BYDV become "adapted" to transmission by a vector species. Undoubtedly there has been considerable doubt as to the identity of some species of Rhopalosiphum commonly found associated with cereal crops and involved in the transmission of BYDV. Hille Ris Lambers (23) claimed that the European and North American apple grain aphids should be regarded as distinct species and the proper name for the later should be Rhopalosiphum fitchii. Orlob (28) also thought R. padi and R. fitchii were readily distinguishable and he referred to the "padi-fitchii complex" although he gave no evidence that he was dealing with both the species.

Bruehl (10) noted that R. fitchii was difficult to establish on cereals in the greenhouse but even so thought the limited success obtained was sufficient to explain the wide spread occurrence of BYDV accredited to this vector in the U. S. A. Orlob and Arny (29) similarly noted that R. fitchii fed poorly if at all on cereals and used this observation to account for the poor ability of some forms to transmit BYDV.

Washington workers viewed their earlier work with misgivings and consequently Bruehl and Damsteegt (12) re-examined the vector specificity of BYDV in Washington and observed that a marked lack of vector specificity was apparently characteristic of BYDV in Washington. However, they did not refute that vector specific strains were present in nature, since they had been more than amply demonstrated.

Bruehl and Damsteegt (12) suggested that "there may be need for further evaluation of the use of the cut-leaf technique" and that "inadequate feeding on excised leaf pieces may partially explain the prevalence of vector specific strains" of BYDV indicated in Rochow's work in New York. Rochow (45) had, however, strongly defended the use of the detached leaf technique. Detached leaves have been used successfully in several virus studies including aphid acquisition of other aphid transmitted viruses such as the potato leaf roll virus (21). Smith (52) also successfully used the detached leaf technique essentially identical with that of Rochow and reported relatively little vector specificity except in Ontario (incidentally adjoining the region of U.S.A. where Rochow reported vector specific strains more common). In fact, MacKinnon (26) had reported that aphids feed better on detached leaves.

For comparison of acquisition and inoculation by different aphid species the detached leaf technique has undoubtedly several advantages. Summarizing some of them, Rochow (45) advocates that "this method reduces chances of variability in the virus source for each aphid species; the chances for accidental mixing of aphids are minimized; it allows easy observation of acquisition feeding; it facilitates acquisition feeding at constant temperature; encourages the use of a control, because aphids from

one colony can be used to infect leaves in many dishes including those containing healthy leaves and those containing diseased leaves." Naturally he believes that the use of detached leaves seems to be a simple, useful and dependable technique and the prevalence of vector specific strains in New York is not the result of a testing technique but instead might reflect the kind of virus that has predominated in that region in recent years.

Watson and Mulligan (69) studied the manner of transmission of some BYDV-isolates in Great Britain and reported that some isolates were transmitted by R. padi and others were not. Sitobium fragariae (Walker), S. avenae (Fab.) and Metopolophium dirhodum all transmitted viruses of both types. They also found that the transmission of a virus by a given aphid species did not interfere with its transmission by another less efficient vector species. They also reported that Neomyzus circumflexus (Buck.) and the CL aphid transmitted some isolates of BYDV.

Virtually nothing is known about the mechanism of vector specificity, the possible existence of additional vector specific strains and the role of such strains in nature. Vector specificity exists in varying degrees of effectiveness ranging from complete exclusion of a species as an active vector to differences in relative efficiency of transmission. Sylvester (61) theorises that vector specificity might be a result of virus inhibition or inactivation within the vector. In the concept of virus inhibition or inactivation, influences of possible insect secretions on the susceptibility of the inoculated host should be included. Although the underlying principles might be similar, regardless of the type of transmission, mechanical or otherwise, the question of vector specificity is

more simply posed by consideration of results obtainable in the transmission of non-persistent viruses.

Black (4) described the specific transmission of two strains of potato yellow dwarf virus by two different species of Agallian leaf hopper. Storey (57) found an active and an inactive race of Cicadulina mbila and was able to change inactive insects to active transmitters by puncturing their gut walls. Storey (57), Fukushi (18), Bennett and Wallace (6) and Black (4) found that leaf hopper species vary genetically in their ability to transmit the different viruses.

Maramorosch (25) reported that transmission of aster yellows virus by Macrosteles fascifrons but not by sibling species M. laevis was probably the most extreme case of vector specificity found as yet. Although many species of Macrosteles and other genera had been reported as vectors of aster yellows-like viruses, no insect other than Cicadellidae had been proven as vectors. Day (14) studied the mechanism of transmission of the potato leaf roll virus by the green peach aphid, Myzus persicae and obtained the first evidence for the occurrence of plant virus multiplication in an aphid.

Maramorosch (25) showed that the latent period in the leaf hopper vector of the aster yellows virus was longer in the insects infected with diluted virus and shorter in insects receiving more concentrated virus. His results suggest that small amounts of virus take a longer time to render the insects infective than larger doses.

Sylvester (61) has recently suggested that aphid transmitted viruses having a persistent relationship should have measurable latent periods provided that experimental conditions could be adjusted to allow

their detection. That BYDV is a persistent virus is quite clear but evidence for a latent period is not clear. Watson and Mulligan (69) explain that this lack of understanding might be due to the use of a long acquisition feeding period. They pointed out that the dominant factor in transmission of BYDV was the time taken to acquire the virus and not the time elapsing between acquisition and transmission. Roohow (46) feels that the demonstration of a latent period in the case of BYDV transmission might explain the aphid-virus relationship. Multiplication of the virus in the vector is one possibility.

MATERIALS AND METHODS

Source of Barley Yellow Dwarf Virus Samples

In 1962, thirty-three samples of plants suspected to be infected with BYDV were collected from different localities in Kansas. These plants were potted, brought to the greenhouse and maintained free of stray aphids by spraying with 0.1% malathion spray.

As soon as the plants were received attempts were made to recover the virus by caging non viruliferous aphids on detached leaves of suspected diseased plants. After a 3-day acquisition feeding period, presumed viruliferous aphids were transferred to Clintland oat seedlings for a 3-day inoculation feeding. Plants inoculated with various isolates were placed in a separate section of the greenhouse and were maintained for future experimental work. These plants were frequently sprayed with 0.1% malathion. The GB was more commonly used in recovery trials. However, the CL aphid and the AG aphid were also used in some cases (Table 3).

Each virus sample collected or received was assigned a code number similar to that used by Allen (1), consisting of essentially 3 parts. The first part, a capital letter, indicates the host from which the virus was recovered (i.e. B for barley, G for grass, O for oats and W for wheat). The second part, an arabic number indicates the locality from which the isolate was collected (i.e. the place from which the first isolate was collected was numbered as 1, the following places were numbered consecutively). The third part, a lower case letter indicates the number of isolates from a particular locality (i.e. the first isolate was designated as a, the second b and so on). As an example, O-8-a denotes a virus isolated from an oat plant, from the eighth locality (Manhattan) from which isolates were collected and the first sample obtained from that particular place.

Raising of Test Plants

Clintland oats was used as the test variety for transmission studies. Seed was obtained from the Kansas Seed Improvement Association at Kansas State University, Manhattan. Plants for experiments were raised in 6-inch pots using a soil mixture of 5 parts of heavy silt loam, 2 parts sand, and 1 part sheep manure. All plants were raised in the starting room of the greenhouse to keep them free from insect infestation. When ready for inoculation, these plants were moved to another section of the greenhouse. Usually 10-12 seeds were planted in each pot and after emergence of the seedlings they were thinned to five per pot. The plants were inoculated at the 3-4 leaf stage. Hyponex was given to all plants every 2 weeks. Plants were watered daily in summer months and as often as needed during winter.

Seed of 4 differential varieties used earlier by Allen (1) was obtained from the Director, Agricultural Experiment Station, University of California, Davis. These differential varieties included 3 barley varieties, i.e. Black Hulless, Atlas 46 and Rojo and 1 oat variety, Coast Black. Seed of each variety was sown in 6-inch pots at the rate of 10 seeds per pot, which were later thinned to 5 per pot after emergence. These were also used for inoculation at the 3-4 leaf stage.

Reno barley was thickly planted in 6-inch pots and the seedlings were used for the rearing of aphid cultures. Plantings were done at regular intervals in order to have enough seedlings available as needed.

Aphid Cultures

The GB, AG, and CL aphid cultures were originally obtained from the Entomology Department, Kansas State University. Stock colonies of these aphids were reared on virus free Reno barley seedlings caged in cylindrical cellulose nitrate cages with nylon tops and maintained at 65-70° F in temperature controlled chambers. The EG aphid culture was obtained from Eldon Ortman[1] and was maintained under similar conditions.

Acquisition and Inoculation Feeding

Unless otherwise specified, all acquisition and inoculation feeding periods were at least 48 hours. As suggested by Rochow (36) aphids were occasionally checked to ascertain whether they were virus-free prior to acquisition feeding.

---

[1]Eldon E. Ortman, Entomologist, ARS, USDA, Brookings, South Dakota.

The detached leaf technique of Rochow (36) was used with slight modifications. Diseased leaves split in pieces, were kept in hinged plastic boxes (3" × 1 1/4" × 2/5") containing moistened germination pads and held at 65-70° F. A single presumably viruliferous aphid, generally late stage apterous, was transferred to each test plant by means of a moist camel's hair brush and each plant was caged in a 100 ml plastic cage (16). After an inoculation feeding of 2-3 days, the aphids were killed by 0.1% malathion spray. Sometimes, early instars of aphids (nymphs) were also included in the inoculation feeding if enough adult aphids were not available. Only those aphids that were actually feeding on detached leaves were used for inoculation feeding, thereby helping to eliminate the possibility of aphids not acquiring the virus.

Types of Cages

Humidified plastic boxes essentially similar to those described by Dody (16) were used as containers for detached BYDV-infected leaves. High humidity was maintained by lining the boxes with a seed germination pad moistened with distilled water. The cages used were hinged plastic boxes (3" × 1 1/4" × 2/5") and proved to be convenient, since the leaves could be well packed inside and would not move during routine placement. Moreover, they were smaller in size and took less space than other cages used. For inoculation feeding, 100 ml plastic test tube cages were used in all experiments and were slipped over each test plant and pushed into the soil.

The seedlings containing aphid cultures were caged in cylindrical cellulose nitrate cages having a top coverdd with nylon fabric and

1-2 holes cut on the sides and covered with nylon or cotton pads. In the beginning of the studies, wooden cages described by Del Rosario and Sill (15) were used for caging the aphid cultures, but proved inconvenient and required extra space. Later on, they were replaced by the cylindrical cellulose nitrate cages (5" diameter, 12" tall).

Greenhouse Facilities

All the experiments were conducted in the north-eastern section of the mosaic greenhouse at Kansas State University. The top ventilators of the greenhouse were covered with double cheese cloth lining on the inside. The greenhouse was regularly fumigated every week with Plantfume 103 (a smoke generator, with active ingredient: 15% tetra-ethyl dithio pyrophosphate) to protect from insects. The daily temperature of the greenhouse during the winter time averaged about 75° F. However, in summer the day time temperature varied considerably, quite often reaching more than 100° F. A heavy coating of white shading compound on the glass and maintenance of high humidity by spraying water on the floor and greenhouse benches was used to help cool the room to some extent. The humidity was not controlled but in general varied inversely with the temperature.

Healthy plants were raised in the starting room of the mosaic greenhouse and this section was equally well protected from insect infestation by means of a strict fumigation schedule.

Temperature controlled chambers with supplemental light, maintained at 65° F, were used for rearing aphid cultures.

RESULTS

Recovery of Virus

Attempts to recover BYDV from diseased samples collected or received were successful in all but two samples (Table 3). Generally GB was used more commonly as the vector for recovery of the virus. However, on some occasions the AG and/or CL aphid was also used. None of the three aphids were able to recover virus from the two isolates, which apparently were carrying no virus. Clintland oats was used as the test plant and the virus isolates were maintained by serial transfer. Since the isolates were collected at random from different localities, the collections gave a good representation of BYDV in the state. It would appear, based upon the collections as well as field observations, that the virus is more prevalent in the eastern half than the western half of the state (Figure 1).

Detached Leaf Technique

In all the recovery work and later in transmission studies, the detached leaf technique was used very successfully. The slight modification of Rochow's technique in using smaller hinged plastic boxes proved more convenient in routine work. The space inside the boxes was quite sufficient to hold the aphids and the leaf pieces on the moistioned germination pad, and the leaves were not displaced in transit. The aphids survived well during the 2-3 days acquisition feeding and leaves remained turgid. Sufficient numbers of aphids were always found feeding on the

Table 3. Recovery of BYDV by aphids from isolates collected in Kansas, 1962.

Date collected	Place collected	Host	Aphid vector			Code No.
			CL	GB	AG	
4-20-62	Gray County	Barley	+	+	+	B- 1- a
4-20-62	Wabaunsee County	Wheat		+	+	W- 2- a
4-23-62	Decatur County	Barley	-	-	-	B- 3- a
2-28-62	Rush County	Wheat	+	+		W- 4- a
5-26-62	Mound Valley	Oats		+		O- 5- a
5-30-62	Ashland farm (near Manhattan)	Oats		+		O- 6- a
6- 3-62	Ashland farm (near Manhattan)	Oats		+		O- 7- a
6- 5-62	Manhattan	Oats	+	+	+	O- 8- a
6- 5-62	Manhattan	Oats	+	+	+	O- 8- b
6- 6-62	Topeka	Oats		+		O- 9- a
6- 6-62	Council Grove	Oats		+		O-10- a
6- 6-62	Near Woodbine	Oats	-	-	-	O-11- a
6- 6-62	Near Woodbine	Oats	-	-	-	O-11- b
6- 6-62	Woodbine	Oats		+	+	O-12- a
6- 6-62	Admire	Oats		+	+	O-13- a
6- 6-62	St. Marys	Oats		+	+	O-14- a
6- 6-62	Junction of Highway 50 and 75	Oats		+		O-15- a
6- 6-62	Herington	Oats		+		O-16- a
6- 6-62	Lyndon	Oats		+		O-17- a
6- 8-62	Republic County	Oats	+	+	+	O-18- a

Table 3. (continued)

Date collected	Place collected	Host	Aphid vector CL	GB	AG	Code No.
6-20-62	St. Marys	Grass		+		0-19- a
6-20-62	Seneca	Oats	+	+		0-20- a
6-20-62	Flush	Oats		+	+	0-21- a
6-20-62	Fairview	Oats		+		0-22- a
6-21-62	East Bennington	Oats		+		0-23- a
6-21-62	West Bennington	Oats		+		0-24- a
6-21-62	Junction City	Oats		+		0-25- a
6-22-62	Hoyt	Oats		+		0-26- a
6-22-62	Holyrood	Oats		+		0-27- a
6-22-62	Holton	Oats		+		0-28- a
6-22-62	Larned	Oats		+		0-29- a
6-22-62	Lincoln	Oats		+		0-30- a
6-24-62	Ashland farm (near Manhattan)	Oats		+		0-31- a
6-24-62	McClure farm (near Manhattan)	Oats		+		0-32- a
6-24-62	Kinsley	Oats		+		0-33- a

AG = Apple grain aphid
GB = Greenbug
CL = Corn leaf aphid

\+ = Virus recovered
\- = Virus not recovered

EXPLANATION OF FIGURE 1

Twenty two Counties (shaded dark) from which BYDV isolates were collected and virus recovered.

Notice that BYDV is more prevalent in the eastern half than in the western half of the state.

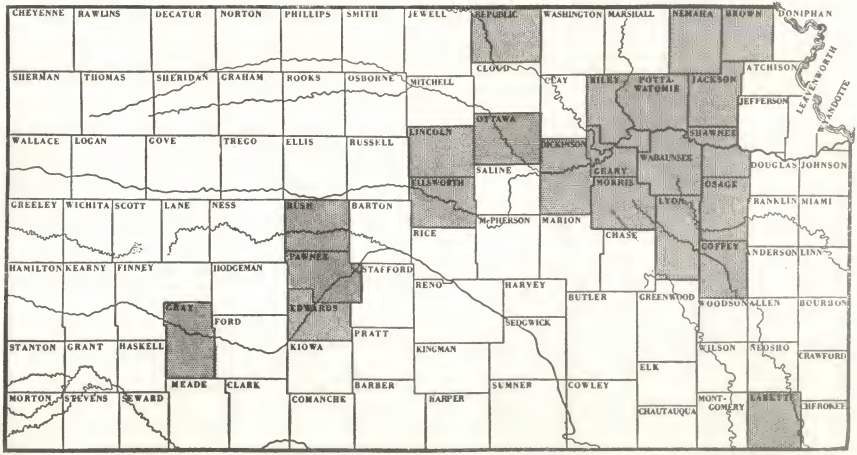
CHEYENNE
RAWLINS
DECATUR
NORTON
PHILLIPS
SMITH
JEWELL
REPUBLIC
WASHINGTON
MARSHALL
NEMAHA
BROWN
DONIPHAN
SHERMAN
THOMAS
SHERIDAN
GRAHAM
ROOKS
OSBORNE
MITCHELL
CLOUD
CLAY
RILEY
POTTA-WATOMIE
JACKSON
ATCHISON
JEFFERSON
LEAVENWORTH
WYANDOTTE
WALLACE
LOGAN
GOVE
TREGO
ELLIS
RUSSELL
LINCOLN
OTTAWA
DICKINSON
SHAWNEE
WABAUNSEE
DOUGLAS
JOHNSON
SALINE
GEARY
MORRIS
OSAGE
ELLSWORTH
LYON
FRANKLIN
MIAMI
GREELEY
WICHITA
SCOTT
LANE
NESS
RUSH
BARTON
McPHERSON
MARION
CHASE
COFFEY
ANDERSON
LINN
RICE
PAWNEE
HAMILTON
KEARNY
FINNEY
HODGEMAN
STAFFORD
RENO
HARVEY
BUTLER
GREENWOOD
WOODSON
ALLEN
BOURBON
GRAY
EDWARDS
FORD
SEDGWICK
PRATT
STANTON
GRANT
HASKELL
KIOWA
KINGMAN
WILSON
NEOSHO
CRAWFORD
ELK
MEADE
CLARK
BARBER
SUMNER
COWLEY
MORTON
STEVENS
SEWARD
COMANCHE
HARPER
CHAUTAUQUA
MONT-GOMERY
LABETTE
BROOKE

Figure 1

leaves to be used in transmission studies. This method also eliminated the possible variation inherent in using different leaves or plants, and the four different boxes containing four aphid species also could be kept under identical conditions.

Transmission by the Greenbugs

Because of the importance of the GB in Kansas, and the circumstantial evidence favoring the role of the GB in local BYDV epidemics, this aphid usually was used for the recovery of virus. Body (16) showed that the GB collected in Kansas was an efficient vector of BYDV in Kansas. Using several Kansas isolates and the GB a very high percentage of transmission was obtained. The results are presented in Table 4. It is interesting to see that an average of 60% transmission occurred when only one aphid per plant was used. Such a high percentage of transmission is direct proof of the importance of the role of GB in field transmission.

Relative Efficiency of Four Aphid Species

After evidence was obtained for the high efficiency of the GB in transmission of Kansas-BYDV isolates (47), it was logical to check the efficiency of transmission of those isolates using four more commonly used known vectors, which are also common in Kansas, namely the AG aphid, the GB, and RG aphid and the CL aphid.

The relative efficiency of the four aphid species in transmitting collections of BYDV appeared to vary considerably. The results are presented in Table 5. It is interesting to observe that the AG aphid appeared to be the most efficient vector (93%). Originally the virus was

Table 4. Transmission of some Kansas BYDV-collections by the greenbug, Toxoptera graminum, (Rond.) to Clintland oat seedlings.

Isolate number	Where collected	No. plants infected	No. plants inoculated	Per cent transmission
B- 1- a	Gray County	19	25	76
W- 2- a	Wabaunsee County	27	40	67
W- 4- a	Rush County	15	25	60
O- 5- a	Mound Valley	10	20	50
O- 8- a	Manhattan	16	35	46
O- 8- b	Manhattan	24	25	96
O- 9- a	Topeka	18	35	49
O-12- a	Woodbine	29	45	69
O-13- a	Admire	20	20	100
O-15- a	Junction Hiway #50 and 75	8	25	32
O-16- a	Herington	23	40	58
O-18- a	Republic County	8	10	80
O-19- a	St. Marys	21	37	57
O-20- a	Seneca	12	25	48
O-21- a	Flush	32	50	64
O-22- a	Fairview	6	20	30
O-25- a	Junction City	19	25	76
O-27- a	Holyrood	21	35	60
O-28- a	Holton	41	50	82
O-29- a	Larned	10	25	40
O-30- a	Lincoln	20	25	80
O-32- a	McClure farm (near Manhattan)	15	25	60
O-33- a	Kinsley	17	25	68
Totals and Average		432	717	60

Table 5. Relative efficiency of four aphid species in the transmission of some Kansas BYDV isolates.

Isolate number	Where collected	Per cent transmission*			
		AG	GB	EG	CL
W- 2-a	Wabaunsee County	82	60	84	4
W- 4-a	Rush County	92	84	76	72
O- 6-a	Ashland farm (near Manhattan)	100	96	100	24
O- 8-a	Manhattan	92	84	64	12
O- 9-a	Topeka	88	92	88	80
O-10-a	Council Grove	100	80	92	24
O-13-a	Admire	96	84	48	40
O-15-a	Junction Hiway #50 and 75	92	72	68	16
O-16-a	Herington	100	84	80	72
O-18-a	Republic County	92	80	64	72
O-19-a	St. Marys	100	96	24	48
O-20-a	Seneca	92	84	64	12
O-21-a	Flush	100	80	100	0
O-22-a	Fairview	90	90	85	30
O-23-a	East Bennington	88	64	72	60
O-27-a	Holyrood	80	64	68	56
O-28-a	Holton	88	76	60	20
O-29-a	Larned	96	48	68	24
O-30-a	Lincoln	84	76	80	68
O-33-a	Kinsley	96	62	92	12
Average		93	77	74	37

AG = Apple grain aphid
GB = Greenbug
EG = English grain aphid
CL = Corn leaf aphid

* = Per cent of transmission based on an average of 25 plants per treatment.

recovered by the GB in most of the cases and transmission with the GB was fairly high. A still higher efficiency of the AG aphid is striking. The GB and the EG aphid gave almost the same percentage of transmission (77% and 74% respectively) whereas the CL aphid appeared to be relatively less efficient (37%). In general, transmission by the CL aphid appeared to be erratic and at least in one particular case the transmission was negative. However, all the four aphid species were quite efficient enough to be important potential vectors of BYDV in the field. No vector specificity was observed unless possibly in the case of the one negative result with the corn leaf aphis.

Statistical analyses of the transmission by four aphids are presented in Table 6. The AG aphid was highly significant at the five per cent level over all the other aphid vectors. No significant difference was observed between the GB and EG aphid but they differed significantly at the five per cent level from the CL aphid in transmission efficiency.

In order to get a general picture of the transmission pattern of the different aphid species, the daily average transmission percentage was plotted and is presented in Figure 2. Transmission by the AG aphid is quite interesting. Besides being a very efficient vector, for unknown reasons, it also produced a shorter incubation period as compared to other aphids. Moreover, the plants infected by the AG aphid showed more pronounced symptoms and severe stunting even with the same virus isolate.

It would appear, therefore, that all the aphids tested were quite efficient in transmission of BYDV isolates in Kansas and potentially important in their ability to transmit BYDV and given favorable conditions could cause epidemics similar to that of 1959.

Table 6. Statistical differences between the four aphid species in their ability to transmit some Kansas BYDV isolates.

Aphid species	Aphid species		
	AG	GB	EG
GB	*		
EG	*	ns	
CL	*	*	*

* = significant difference at 5% level
ns = non significant difference

EXPLANATION OF FIGURE 2

Characteristic incubation periods and the relative efficiency of 4 aphid species in transmitting 20 Kansas BYDV isolates.

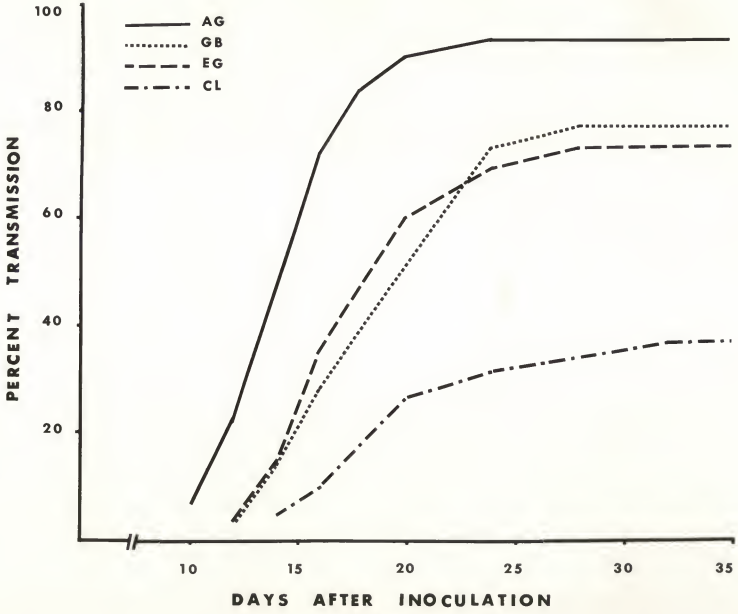
AG
GB
EG
CL
100
80
60
40
20
PERCENT TRANSMISSION
10
15
20
25
30
35
DAYS AFTER INOCULATION

Figure 2

Use of Differential Varieties in Strain Identification

Strains are known to occur in BYDV collections. Since no vector specific strain was observed for certain, it was logical to use the differential host reactions as a basis of strain identification. In an attempt to identify the strains of this virus, four differential varieties, (3 barley: Rojo, Black Hulless, Atlas 46 and one oat: Coast Black) used earlier by Allen (1) were used in these experiments. The AG aphid was used regularly to transmit the BYDV to these varieties. The results of the reactions of several isolates to these differential varieties are presented in Table 7.

It was interesting to see that several isolates behaved quite similarly and based on an average of three trials, the isolates were grouped into three types (Table 8). The strain types are coded on the basis of presence (+) or absence (-) of symptoms on Coast Black oats, Black Hulless barley, Atlas 46 barley and Rojo barley, respectively. Thus ++++ designated as Type 1 showed symptoms on all four varieties, whereas +++- designated as Type 2 showed symptoms on all but Rojo barley. The isolates which showed variable reactions have been included in Type 3. Types 1 and 2 definitely show consistent symptoms whereas isolates grouped under Type 3 need further confirmation and clarification. Instead of classifying them as strains on the basis of degree of stunting, more emphasis was laid on the symptom expression on the differential host and the isolates were grouped in "types". However, in general, for the three types of BYDV differentiated all the four aphid species tested were efficient enough to be economically important.

Table 7. Reaction of various collections of Kansas BYDV isolates on four differential barley and oat varieties using the apple grain aphid, Rhopalosiphum fitchii, as vector.

Isolate number	Collected from	Trial number	Differential varieties			
			Coast Black	Black Hulless	Atlas 46	Rojo
B-1-a	Gray County	1	+++++	+++	+++	-
		2	+++	+++	++++	-
		3	++++	++++	+++	-
W-2-a	Wabaunsee County	1	+++++	++++	++++	+++
		2	++	+++	++++	+
		3	++++	++++	++	+
W-4-a	Rush County	1	++++	++++	++++	-
		2	+++	++++	++	-
		3	+++++	+++++	+	-
O-5-a	Mound Valley	1	+++++	+++++	++++	++
		2	++++	++++	++++	++
		3	++++	++++	++	++
O-6-a	Ashland farm (near Manhattan)	1	++++	++++	+	-
		2	++++	++++	+++	-
		3	++++	++++	++++	-
O-8-a	Manhattan	1	+++++	+++++	++++	-
		2	+++++	++++	+++++	-
		3	++++	++++	++	-
O-9-a	Topeka	1	+++	++++	++++	-
		2	++++	+++++	+++++	++
		3	++++	+++++	+++++	++
O-10-a	Council Grove	1	++++	+++++	++++	+
		2	++++	+++++	+++++	++
		3	++++	+++++	+++++	++
O-12-a	Woodbine	1	+++++	+++++	++++	+
		2	++++	+++	++	++
		3	++++	++	++++	++
O-15-a	Junction of Hiway #50 and 75	1	+++++	++++	++++	+++
		2	+	+++	++++	+
		3	++++	+++	++++	+
O-16-a	Herington	1	+++++	++++	+++	-
		2	+++++	++++	++	-
		3	+++++	+++	+++	-
G-19-a	St. Marys	1	+++++	+++++	++++	-
		2	+++++	+++++	+++++	-
		3	++++	+++	+	-

Table 7. (continued)

Isolate number	Collected from	Trial number	Differential varieties			
			Coast Black	Black Bulless	Atlas 46	Rojo
0-20-a	Seneca	1	+++	++++	+	+
		2	++++	++++	++++	++
		3	++++	++++	++	+++
0-21-a	Flush	1	+++++	+++	+++	+
		2	+++++	++++	++++	-
		3	+++	++++	++	++
0-22-a	Fairview	1	+++	++++	++++	+++
		2	+++++	+++++	+++++	+++
		3	++++	+++++	++++	++
0-23-a	East Bennington	1	++++	+++++	+++++	++++
		2	++++	++++	++++	++
		3	++++	++++	++	++
0-25-a	Junction City	1	+++++	++++	++++	-
		2	++++	++++	+++	-
		3	++++	++++	++	-
0-27-a	Holyrood	1	-	++	++	-
		2	++++	++++	++	-
		3	++++	++++	+++	-
0-28-a	Holton	1	+++++	++++	++++	+
		2	+++++	+++++	++++	+
		3	++++	+++	++	++
0-29-a	Larned	1	++++	+++	+++	+++
		2	++++	++++	++++	++
		3	+++++	+++++	+++	++++
0-30-a	Lincoln	1	++	+++	-	-
		2	++++	++++	++	++
		3	+++	+++	++	+
0-32-a	McClure farm (near Manhattan)	1	+++++	++++	++++	++
		2	+	+++	+++	+
		3	++++	+++++	++++	+++
0-33-a	Kinsley	1	+++++	+++++	+++++	+
		2	++++	+++	+++	++
		3	+++	+++	+++	++

Each + represents one plant showing symptoms out of five inoculated, - means no plant showed symptoms.

Table 8. Isolates arranged according to their average reaction on the four differential barley and oat varieties, using the apple grain aphid, Rhopalosiphum fitchii (Sand.) as vector.*

Type				Isolates
		No. 1		
CB	BH	At46	Rojo	W-2-a; O-5-a; O-10-a; O-12-a;
+	+	+	+	O-15-a; O-20-a; O-22-a; O-23-a;
				O-28-a; O-29-a; O-32-a; O-33-a.
		No. 2		
CB	BH	At46	Rojo	B-1-a; W-4-a; O-6-a; O-8-a;
+	+	+	-	O-16-a; G-19-a; O-25-a.
		No. 3		
Reaction variable				O-9-a; O-21-a; O-27-a; O-30-a.

*Reactions based on symptoms produced on at least 1 plant in each variety, in 3 trials.

Differential varieties:

CB = Coast Black
BH = Black Hulless
At46 = Atlas 46
Rojo = Rojo

DISCUSSION

Prior to this study circumstantial evidence suggested that the GB might be the most important vector of the BYDV in this part of the country. Although Rochow (39) found the GB as the least efficient vector in New York, several workers successfully used this aphid in transmission studies. Co-existence of the GB in large populations in several areas suffering from severe damage from BYDV in 1959 and in later years added further circumstantial evidence that GB was an important vector. However, most of the reports on aphids that are responsible for transmitting the BYDV are based on field observations, and much evidence for the role of different aphid species is indirect. Many of these observations are based on the assumption that the most common aphid species is the most important vector. Since this assumption is known to be false in many cases involving other aphid transmitted virus (7, 21, 55), it could also be misleading in the case of BYDV. In Maine, Stetson (56) found that even though the CL aphid was the predominant one in certain areas, yet the most efficient vector was the EG aphid. Moreover, the major aphid vectors are known to be different in different farming areas from year to year or season to season. In 1958, in Ontario, Canada, R. padi appeared to be the most important vector. In 1959, in the same region the EG aphid was probably the most important (62). Similarly, Rochow (44) reported that while the EG aphid was a predominant vector of BYDV in a field near Cornell in 1959, the next year the CL aphid appeared to be more important. The sequence of occurrence of different vector species during any one season might be a further complicating factor.

In Kansas, Sill et. al. (49) believed that the GB was, in all probability, the most important vector in this region. Medler (24) reported

trapping the GB in large numbers in 1962 at Manhattan and further substantiated this circumstantial evidence. The AG aphid, the CL aphid and the EG aphid were also trapped but were generally rare. A few direct attempts have been made to determine which aphids are important vectors in nature (29, 56). Direct evidence for the occurrence of naturally viruliferous aphids was obtained by Slykhuis et al. (62) in Canada, and Jedlinski and Brown (20) in Illinois. Many more such direct tests are needed before the exact role of the different aphid species can be evaluated.

Dody (16) reported that the GB were quite efficient in Kansas in transmission of a strain of BYDV obtained from New York. Rochow's report (41) on physiological specialization amongst the GB might explain some of his previous negative results. The GB was used successfully in the recovery trials of BYDV-isolates in Kansas and has been proven to be a very efficient vector of the Kansas BYDV-isolates tested. Saksena et al. (47) reported the efficiency and importance of the GB in field transmissions of BYDV.

Not all aphid species are of equal importance in their efficiency to transmit BYDV. It was interesting to find the AG aphid to be the most efficient vector in this study. All four aphids used were very efficient and certainly capable of causing epidemics under favorable conditions. The transmission by the AG aphid is especially interesting in as much as it was very highly significant in its transmission efficiency as compared to the other aphid species. The incubation period, averaging about 10 days was, for some unknown reason, also shorter and the symptoms were more pronounced. Washington workers (8) reported that the AG aphid was very important in that area. Oswald and Houston (32) in their initial studies also reported the AG aphid as the most efficient vector (they referred to

the aphid as R. prunifoliae). Slykhuis et al. (62) and Toko and Bruehl (64) also showed that the AG aphid was a more efficient vector of BYDV than the EG aphid and produced a more severe disease. Rochov (42) and Bruehl and Toko (8) have reported strains that are transmitted by several aphid species but with varying efficiency. This is important since it proves that the more predominant aphid species need not necessarily be the most important vector. Even the species which might occur in small numbers could be quite important as far as the transmission of BYDV and subsequently be important in its spread in the field.

As Bruehl (11) has pointed out, the bulk of the isolates in many areas of the United States were non specific and could be readily transmitted by more than one aphid species. No vector specificity has been observed definitely as yet in this study. However, the CL aphid was extremely erratic as a vector of the different isolates and at least in one case did not transmit a specific isolate. Saksena et al. (48) reported earlier the differential transmission of BYDV by four biotypes of the CL aphid. The CL aphid culture used in this study was a mixture of biotypes and it could have been possible that in this particular case the aphids used for transmission mainly consisted of the biotypes which were least efficient or inactive. However, complete failure of the CL aphid to transmit this particular isolate is striking and needs further study to confirm whether or not we have a vector-specific isolate. Prevalence of vector-specific strains in New York and their rare occurrence in Washington led Bruehl and Toko (8) to suspect the presence of either regional virus complexes, vectors or both. Bruehl (10) suggested that abrupt fluctuations in aphid population might place selection pressure on the virus towards versatility in vector relationship. The apparent lack of vector specificity

in the Kansas area represents greater virus and vector adaptability and should favor increasing chances of survival and spread of BYDV in the field.

It would appear quite interesting to explore the possibilities of using several aphid strains or biotypes to see if the virus strains reported elsewhere remain vector specific. Several kinds of specializations among the vectors are known and their ability to transmit a strain of BYDV varies with different biotypes or collections. Stubbs (58) found that individual cultures of Myzus persicae (Suls.) varied in their ability to transmit the virus and postulated that inactive insects might occur more frequently than is presently realized and could account for much of the variability reported. Storey (57) also reported that the presence of active and inactive insects in certain collections might give varying results. Rochov (42) found physiologically specialized forms of GB which differ in their ability to transmit BYDV and could partly explain his previous almost negative transmission results of BYDV in New York with the same aphid. Future work should involve not only known and unknown strains of virus but known and unknown strains or biotypes of the aphids to obtain more definite knowledge concerning relationships involved.

Specificity among insect vectors of plant viruses has received considerable attention and has been regarded as an important fundamental relationship. It has been shown that there are varying degrees of specificity and vector efficiency among aphid and leaf hopper vectors of plant viruses. This ranges from lack of ability to transmit through various degrees of efficiency to highly efficient transmission. The value of vector specificity is quite important if it is absolute. But in general, it has been reported to be relative as in the case of BYDV, but rare transmission of a virus by the 'non vector' could really be important. It shows

the danger of using only one aphid species to confirm the presence of the disease in areas where the predominating virus strains are unknown.

The details of acquisition and inoculation feeding periods have not yet been fully explored and times ranging from five minutes to several hours have been reported to be sufficient to assure acquisition and infection. Rochow (40) however, recommended that at least 24 hours of acquisition and 24 hours of inoculation feeding be given in routine work to get successful results. Different workers have used different periods of time for feeding and inoculation and it might be hard to compare the results which have been obtained under different sets of conditions. In these studies, acquisition and inoculation periods of at least 2-3 days have been used with success. Smith and Richards (54) have recently pointed out that the acquisition feeding period for a little longer time was more effective and has proposed a dosage concept to support it. Certainly long acquisition and inoculation feeding periods are the rule of nature in the field.

All the aphids tested in this study were efficient vectors of BYDV-isolates even when only one viruliferous aphid per plant was used. It would seem that a large mobile aphid population in the field of any aphid tested would easily cause a severe outbreak under favorable conditions.

Recently Washington workers (12) suggested a need for further evaluation of the use of the detached leaf technique. They suspected that inadequate feeding on excised leaf pieces might partly explain the prevalence of vector specific strains of BYDV in New York. Several workers (16, 36, 47, 52) have used this technique successfully. Smith (52) also used this technique but did not report the prevalence of vector specificity except

for one area which was adjacent to the place where Rochow (36) had earlier reported the prevalence of vector specific strains. It would appear that it is the kind of virus that is prevalent in a certain area, rather than the technique which gives varying results. In fact, MacKinnon (26) reported that aphids seem to prefer feeding on detached leaves better than the intact leaves. The several advantages of the detached leaf technique have been emphasized by Rochow (45). In these studies here, the detached leaf technique has been used very successfully but no definite vector specificity has been observed. This confirms Rochow's idea that vector specificity reported in his studies was not the result of faulty technique used, but instead the kind of virus in that area. The vector specific strains do apparently exist and their presence seems to have been amply demonstrated. It seems though that here in Kansas we have virus strains which are all fairly efficiently transmitted by several aphid species. However, variation amongst the vectors themselves has been reported and evidence has been presented that different strains of aphids differ in their ability to transmit BYDV strains (8, 27, 29, 34, 39).

Since no vector-specific strain was definitely observed, it was logical to use host reactions as a method of strain separation. Bruehl and Toko (8) described a Washington strain on the basis of symptom expression on different cereal hosts. Takeshita (63) separated two strains of virus by differences in virulence. Allen (1) tested several isolates which he classified as 16 strains based on the absence or presence of discoloration and the extent of stunting of four differential varieties. These 16 strains were grouped in seven types depending on whether or not discoloration was induced in the four varieties. On the basis of symptom expression, the

Kansas BYDV-isolates were grouped in three Types. The Type 1 and 2 would be comparable to Type 1 and 2 of Allen (1). The results here would confirm Allen's result that Type 1 and 2 are more predominant types, and are commonly distributed. The Type 3 described in this study comprised the isolates whose reactions varied considerably and it would seem premature to conclude as to which 'type' they actually belong. Allen (1) distinguished 16 strains on the basis of the tolerance or susceptibility of a certain host as indicated by the degree of stunting. Although his results were statistically significant, it seems rather difficult to judge the exact degree of dwarfing. In fact, he also observed that stunting incited by one isolate on Coast Black oats ranged considerably; it incited no stunting in one trial, whereas it incited up to 60 per cent stunting in subsequent trials. It would not be surprising if such variation could occur in case of other hosts as well. Consequently, the validity of a rating system based on stunting is questionable. It would seem, therefore, more logical to emphasize the 'types' of BYDV encountered which would eventually serve as more useful tools for the plant breeder. The variability of stunting by any one isolate could also be due to mixture of strains, variations in plant genetics or even to environmental conditions.

Many of the differences among isolates of BYDV are so marked that characteristic isolates have been called strains. However, the results of cross protection tests which are widely used to determine strain relationship fail to support the position that isolates are closely related strains (8, 38). The issue will remain unsolved until chemical, physical and serological criteria can also be applied to the problem. Basic studies

on the nature of the virus itself and relationships amongst the isolates have not been made because the virus is only aphid transmitted and attempts to transmit BYDV from plant to plant by mechanical means have failed (9, 34).

SUMMARY

All but two of the 33 presumed barley yellow dwarf virus (BYDV) isolates collected in 1962 were carrying the virus. BYDV was more predominant in the eastern half than in the western half of the state.

Initial transmission studies by the greenbug (GB), Toxoptera graminum (Rond.) showed that it was a very efficient vector and is certainly important in field transmission. However, a comparative test using 4 aphid species (the apple grain (AG) aphid, Rhopalosiphum fitchii (Sand.), the greenbug (GB), the English grain (EG) aphid, Macrosiphum granarium (Kirby) and the corn leaf (CL) aphid, Rhopalosiphum maidis (Fitch)) revealed that the AG aphid was the most efficient vector (93%) and was significantly different at the 5% level from the other 3 aphids. The incubation period averaging about 10 days was, for some unknown reasons, also shorter and the plants showed more pronounced symptoms and stunting. No significant difference was observed between GB and EG aphids (77% and 74% respectively) but they were significantly different at the 5% level over the CL aphid (37%) which incidentally was quite erratic in its transmission and in one case did not transmit the virus at all. However, the 4 aphid species tested were efficient enough to cause epidemics under favorable conditions. No definite vector specificity was observed.

Using 4 differential varieties (3 barley varieties: Black Hulless, Atlas 46 and Rojo and 1 oat variety: Coast Black) an attempt was made to identify the strains of BYDV in Kansas. The isolates tested were grouped in three types: Type 1 includes those showing symptoms on all the four varieties and Type 2 consists of those showing symptoms on all varieties except Rojo. Some isolates showed variable reactions and have been tentatively grouped as Type 3. These need further confirmation.

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RELATIVE EFFICIENCY OF FOUR APHID SPECIES IN TRANSMISSION OF BARLEY YELLOW DWARF VIRUS AND USE OF DIFFERENTIAL VARIETIES IN STRAIN IDENTIFICATION

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Thirty-three barley yellow dwarf virus (BYDV) isolates collected in Kansas during 1962 were tested for the presence of virus. All but two samples were carrying virus which was recovered by using the greenbug (GB), Toxoptera graminum (Rond.) as vector. The corn leaf (CL) aphid, Rhopalosiphum maidis (Fitch) and the apple grain (AG) aphid, Rhopalosiphum fitchii (Sand.) were also used in some cases with success. The BYDV was more prevalent in the eastern half than in the western half of the state.

Since circumstantial evidence indicated that the GB might be the most important vector in this part of the country, transmission studies using the GB as vector were conducted. Using 20 virus isolates in transmission tests with 717 plants an average of 60% transmission was obtained. Such a high percentage of transmission, when only one viruliferous aphid was used per plant, is quite efficient. It would, therefore, seem certain that a large mobile population of the GB would be potentially dangerous in the spread of BYDV in the field.

The relative transmission efficiency of 4 species of aphids was tested for the BYDV-isolates. No definite vector specificity was observed. The AG aphid was the most efficient vector (93%) and was significantly more efficient at the 5% level than the other 3 aphid species. The incubation period, averaging about 10 days was, for some unknown reasons, also shorter and the symptoms produced on the plants were also more severe. The English grain (EG) aphid, Macrosiphum granarium (Kirby), and the GB also were very efficient in their transmission (77% and 74% respectively). No statistical difference was observed between these aphids. However, they were significantly different at the 5% level from the CL aphid, which was the least efficient vector (37%). The transmission by the CL aphid, in general, was

somewhat erratic and in one particular attempt it did not transmit the virus at all. Whether this is a case of relative vector specificity remains to be seen. All of the 4 aphid species tested were efficient enough to cause BYDV-epidemics under favorable conditions in the field. The lack or scarcity of vector specificity in this region represents greater virus and vector adaptability and should favor the chances of virus survival and severity.

The detached leaf technique adopted from Rochow and slightly modified for convenience was used very successfully. In these studies, although the detached leaf technique has been used regularly, no definite vector specificity was observed. This would confirm Rochow's idea that the vector specificity has nothing to do with the kind of technique used for acquisition feeding. It would seem that it is instead related to the kind of virus that is prevalent in a certain area.

Attempts were also made to identify strains of BYDV in Kansas. Since no vector specific strain could be isolated, a logical method was to use the differential varieties used earlier by Allen. Twenty isolates were tested for their reaction to the differential hosts, i.e. 3 barley varieties, Black Hulless, Atlas 46 and Rojo and 1 oat variety, Coast Black. Based on the presence or absence of symptoms on the host, these isolates were grouped into three types. Type 1 included isolates which showed symptoms on all four host varieties whereas Type 2 includes the isolates which showed symptoms on all the differential hosts except Rojo barley. The isolates whose reactions varied were temporarily grouped in Type 3. These need further confirmation and study. Allen classified these

different types into 16 strains and based his rating system on the degree of stunting. But in these studies more emphasis was given to host range rather than the degree of stunting. It appears that we have in Kansas the two types of BYDV comparable to Type and 2 of Allen which incidentally were the more predominant types.